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PATHOLOGIC HISTOLOGY OF APPLE BLOTCH

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WITH PLATES XXX AND XXXI AND FIVE FIGURES IN THE TEXT

The history of the Phyllosticta apple blotch, caused by *Phyllosticta solitaria* E. & E., began with collections of this disease by Underwood (7) from Crawfordsville, Indiana, in 1893, on the leaves of the native American crab apple (*Pyrus coronaria* L.) and by Clinton (1) from southern Illinois in 1902 on the fruit of the commercial apple (*Pyrus Malus* L.). In 1920 Roberts (3) presented a brief account of the pathologic histology of apple blotch cankers. To the knowledge of the writer this appears to be the only published account on this subject. The object of this paper is a further and more extensive consideration of the pathologic histology of this disease. The main results of the investigation, already published in abstract form, will appear in print subsequently.

When this study was undertaken it was hoped to determine the mode of entrance of the fungus into the healthy tissues and the morbid changes in the tissues immediately following infection. Unfortunately, these points have not been solved by this study for reasons which may be briefly enumerated here. Spore production in culture of species of the genus Guignardia, to which, no doubt, *Phyllosticta solitaria* belongs, appears to be a rare occurrence, this being decidedly so of *Phyllosticta solitaria*. In the absence of a supply of spores from pure culture, artificial inoculations of the host with spores from this source were not possible. Efforts were made to inoculate the host with spores from apparently mature fruiting bodies obtained from lesions on the host but the spores from these sources when available and when needed generally failed to germinate. Scott and Rorer (5) (6) report having obtained successful artificial inoculation in the orchard with spores obtained from natural sources, but their results are open to criticism since the inoculated parts were exposed to natural infection and spores from pure culture were not used. Roberts (4), however, established the pathogenicity of the fungus with spores from pure culture. The accounts of his results are presented rather briefly without affording any definite conclusions regarding spore production in culture or regarding infection. No one has yet determined the manner in which infection takes place. The problem of spore production in culture and artificial infection with spores warrants investigation. In view of the writer's failure to obtain artificial infection, certain points of interest in the pathologic histology of the disease could not be determined. This account, there-

fore, is largely based on the gross histology of affected tissues as revealed in microscopic sections.

IN THE TWIGS

The anatomy of the normal bark of one year old apple twigs in transverse section reveals the cuticle, epidermis, cork layers, a compact, rather broad band of collenchyma of four tiers of cells and a loose spongy parenchyma with many intercellular spaces (Plate XXX, fig. B). Strands of sclerenchyma are located deep in the spongy parenchyma, that is, in the pericyclic region, and beyond them are evident the medullary rays. Within the sclerenchyma and between it and the cambium is the phloem. Frequently in the young twigs individual strands of sclerenchyma are united to form an unbroken irregular sheath of sclerenchyma around the phloem and the terminations of the medullary rays.

A transverse section through diseased bark reveals necrosis extending to a point midway between the epidermis and the cambium. The diseased tissues are separated from the healthy, directly beneath the sclerenchyma,

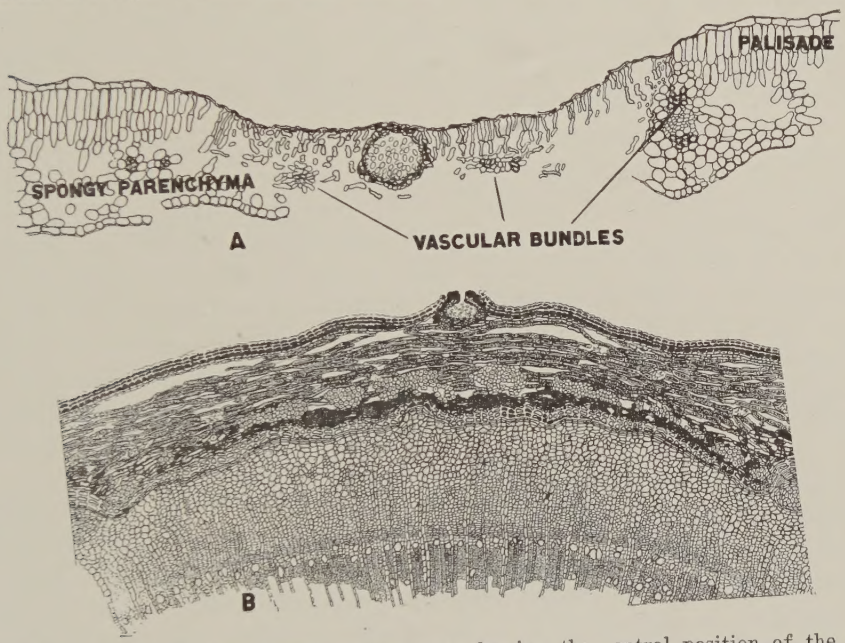

FIG. 1. A. Section through a leaf spot showing the central position of the pycnidium, the dead palisade cells hanging loosely from the collapsed epidermis, the mass of dead parenchyma cells around the vascular bundles, and the compact mass of living cells across the mesophyll at the vascular bundle. B. Section through a canker on a water sprout showing the necrotic region on the outside, the cork cambium, and the dense underlying region of small parenchyma cells (phelloderm). From material collected November 10, 1919.

by a compact cork layer of two to three tiers of cells (Fig. 1, B). Directly outside of this cork cambium is a compact sheath of dark colored cells. The formation of periderm by the cork cambium occurs rapidly. Phellem production is limited to a few layers of cells while, to the inside, phelloderm production is very marked (Plate XXX, fig. A and C). Coincident with the formation of periderm the cambium layer is stimulated in places to rapid formation of parenchyma (Plate XXX, fig. A) instead of normal phloem. The phelloderm is composed of small round cells arranged in compact radial columns (Fig. 1, B) and the medullary rays which extend out through the phelloderm to the cork cambium are distorted and composed of small cells.

The mycelium is intercellular and generally present outside of the abscission layer and its development appears to be most extensive in the collenchyma. The fungus does not come into contact with the phloem, medullary rays and cambium although these tissues appear to be markedly affected indirectly as the result of the necrosis of the outer cortex. Between the lower tiers of cells of the collenchyma and the outermost tiers of cells of the spongy parenchyma, small knots of mycelium are evident, these later developing into pycnidia or pycnosclerotia according as they are formed early or late in the season.

In August and September when the new cankers first became evident and during the course of the following dormant season the diseased parenchyma cells outside of the periderm still appear to be living. The growth of the cankers is to a large extent inhibited during the dormant season. Cracks are formed in the fall especially along the longer margins of the cankers, which in transverse section appear just outside the periderm (Plate XXX, fig. C). The growth of the fungus occurs actively again early in the spring and, as a result, the cells within the invaded region are killed. The presence of the protective layer and the complete isolation of the necrotic tissues, with the resulting exposure of these tissues to desiccation, probably also plays an important part in the death of the cells.

New cankered areas appear along the margins of the old cankers. Whether the fungus is already present in the parenchyma beyond the cork cambium and merely continues its growth when favorable conditions permit or whether it penetrates the periderm and infects the healthy tissues beyond it early in the season has not been determined. In Illinois the growth of the fungus and the formation of new cankered areas appear to be most marked early in the spring and early in the fall. The necrosis of the outer cortex and the formation of cork cambium layers below the sclerenchyma continue for some years. The cankered areas become rifted at the margins (Plate XXXI, fig. D) and by being marked with different shades of brown appear

distinct from other portions of the canker. The dead tissues are gradually exfoliated (Plate XXXI, fig. B) thus exposing the cork cells or phellem. The increase in the number of cells between the cork cambium and the cambium as the result of the activity of these meristematic tissues and their growth in size and differentiation continues until the wound is repaired.

It is apparent from the foregoing account and the study of plate XXX, fig. A and figure 1, B that the fungus may be readily removed from the bark with a knife by cutting away all of the discolored tissues. This exposes the fresh healthy phloem between the abscission layer and the cambium and the rapid formation of new phloem by the cambium eventually repairs the wound.

IN THE PEDICELS

A transverse section of the cortex of a normal apple pedicel shows on the outside a thin cuticle, then a narrow epidermis, surmounting a much broader layer of collenchyma of about four to five tiers of cells and next spongy parenchyma of large cells and intercellular spaces. Beyond the parenchyma are regions of sclerenchyma which are united more or less into broad irregular bands around the phloem and the terminating cells of the medullary rays.

The pathologic histology of the pedicel in transverse section generally appears to be similar to that of the twigs (Fig. 2). The necrosis of the pedicel extends midway into the parenchyma. Here, however, the protective layer forms outside of the sclerenchyma sheath rather than inside as was found to be the case in the twigs. The cells of the protective layer are commonly round or broad and elongate with truncate ends exposed to the necrotic area and their walls and contents are brown. There is no indication of the formation of phelloderm or of the presence of a cork cambium. It appears that individual cells of the spongy parenchyma between the protective layer and the sheath of sclerenchyma have divided to account for hyperplasia in this region. Outside of the protective layer the tissues are collapsed and desiccated. This layer prevents drying, affords mechanical protection and hinders the invasion of the underlying parenchyma by the pathogene.

The mycelium is generally distributed throughout the necrotic tissue although most extensively developed in the collenchyma where necrosis occurs at a more rapid rate than in the parenchyma directly beyond. Pycnidia are formed between the upper tiers of cells of the collenchyma or directly below the epidermis.

IN THE FRUIT

The healthy apple when studied in transverse section shows a thick cuticle lying over an epidermis composed of irregular polygonal cells. Below the epidermis lies the hypodermis of about 4 to 5 tiers of cells. The ultimate branchlets of the vascular bundles approach the hypodermis. Beyond, extending to the primary vascular bundles, is a much broader region of spongy parenchyma which with the hypodermis comprise the "cortex" or the outer pericarp. Ten primary vascular bundles lie in a circle about midway between the central cavity and the epidermis and mark the division between the cortex and the "pith" or inner pericarp. The pith is comparatively narrow, composed of spongy parenchyma extending to the endocarp. It is distinguishable from the cortex by the absence of vascular tissue and by the longer, narrower cells.

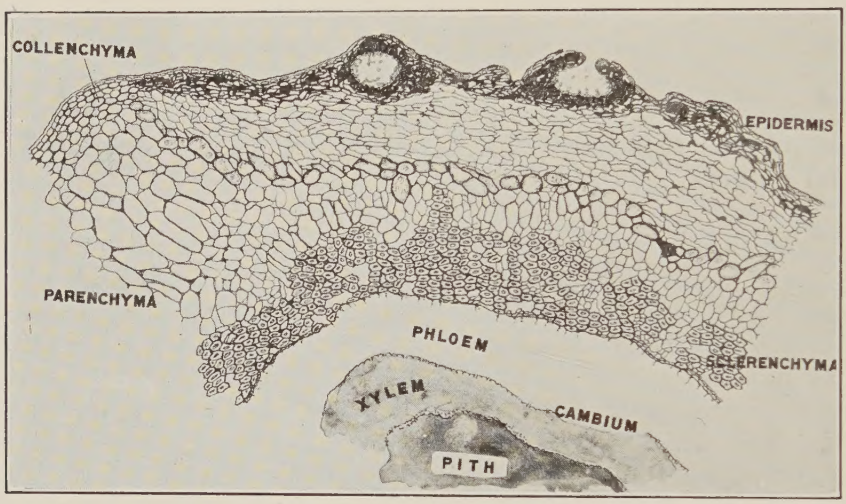

FIG. 2. Section through an affected pedicel. The living and dead areas are separated by a definite protective layer of dark colored cells; below in the parenchyma tissue and outside of the sclerenchyma the tissue is compact and composed of small irregular shaped cells.

The different varieties of apples usually manifest different symptoms of apple blotch which may be classified according to their external appearance as: the pitted; the blistered; and the fringed blotch types. The lesions of the fringed blotch type are radiate and feathery with fringed margins. This type may be found on the Northwestern Greening, Maiden Blush, Yellow Transparent, and Duchess varieties. The pitted type is characterized by sunken areas with definite and distinct borders; it appears to be

common on the Jonathan, Missouri Pippin, Ben Davis, Arkansas Black, and McAfee varieties. This type is excellently illustrated by Lewis (2). The fringed blotch type precedes or sometimes surrounds the pitted areas as they enlarge. The occurrence of deep rifts across the blotches appears to be common to the fringed blotch type. In contrast to the hard and superficial qualities of the lesions of the fringed type, the lesions of the pitted type are deeper and the tissue is soft although also dry. The early stages of apple blotch are usually all of the fringed type; later as the fruit matures some portions become pitted while other portions remain distinctly feathery and spreading. The third type is manifested in the form of blisters with partly cracked margins. This type appears to be common on the May of Meyers and the Benoni.

From a histological study of the affected tissues of apples exhibiting these types of blotch it appears that the reaction of the host cells to the invading fungus is responsible for differences in the symptoms. These different types of apple blotch appear to be quite characteristic of certain varieties. It seems logical to believe that they are the result of differences in the growth of the apple peculiar to the variety rather than of different strains of fungus.

a. *Fringed Type of Apple Blotch*

In the fringed type of apple blotch, necrosis is confined to the epidermal and hypodermal cells. The spongy parenchyma cells directly beyond apparently are not affected. Growth of the apple below the affected portion is stunted while the tissues around the necrotic region continue growth and, as the result, create opposite stresses. The necrosis of the cells of this outer region of the fruit also unduly exposes the tissues to drying. As the result of these abnormal conditions cracks form across the surface of the blotch. The crack at first is broadly wedge-shaped and extends into the hypodermis. In the lower hypodermis the cells are brown and are arranged very compactly. In the spongy parenchyma broken cell walls and radially elongate cavities are evident and ultimately, the crack extends deep into the pith. The cells of the spongy parenchyma adjacent to the rifts become torn and radially elongate, and greatly distorted about the bundles.

b. *Blistered Type of Apple Blotch*

The lesion is quite superficial, confined to the hypodermis and epidermis and limited radially by the formation of distinct periderm directly below the hypodermis (Fig. 3). Directly outside of the periderm is a protective layer of compactly arranged round, dark colored cells. The abscission region inhibits the progress of the fungus radially into the spongy paren-

chyma, although tangentially the fungus grows slowly along the hypodermis causing necrosis of the cells. The raised blistered blotches result from active growth of the cork cambium, giving rise to a broad, compact mass of small cells, the phelloderm, arranged in radial columns. The amount of phellem produced is comparatively insignificant. The epidermis is sometimes broken at the margins of the blotches. As desiccation progresses, the collapsed, discolored cells become more or less separated from the abscission layer.

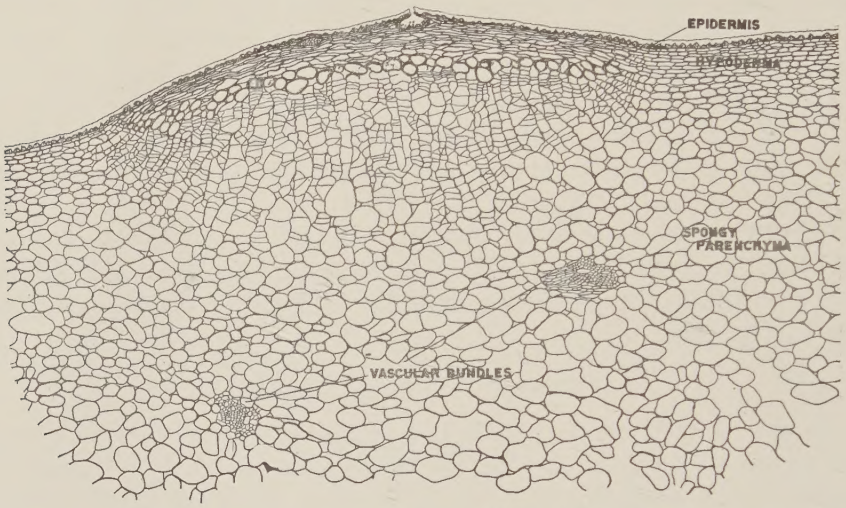

FIG. 3. Section through an apple affected with the blistered type of apple blotch showing the distinct protective layer of large, round, dark colored cells and the periderm layer dividing the living and dead tissues. The dense columnar arrangement of the cells of the phelloderm and their marked multiplication in number is very distinct of this type of apple blotch.

c. *Pitted Type of Apple Blotch*

A marked contrast between the pitted type and the blistered type of apple blotch is indicated by the absence of a distinct abscission layer between the dead and living tissues and in the presence of large cavities in the cortex directly beyond the hypodermis (Fig. 4). Necrosis extends deep into the cortex below the outer vascular bundles. The epidermal and hypodermal cells are collapsed, these tissues together forming a dense, collapsed, region. Below in the spongy parenchyma the cell walls are torn apart, and in the corners of the dead cells masses of starch grains are evident, their formation being the result of disease.

The dead pitted region is sometimes definitely surrounded by normal spongy parenchyma, hypodermal and epidermal cells. Usually the pitted

area is surrounded by an area of blotch of the fringed type, in which case the hypodermis and the epidermis only are affected. The pitted region is dry but soft, due to the large open spaces in the cortex.

The intercellular mycelium is extensively developed directly beyond the epidermis and extends loosely among the cells of the hypodermis and partly into the spongy parenchyma. It is entirely absent deep in the affected cortex. The pycnidia form directly below the epidermis and commonly between the upper tiers of cells of the hypodermis.

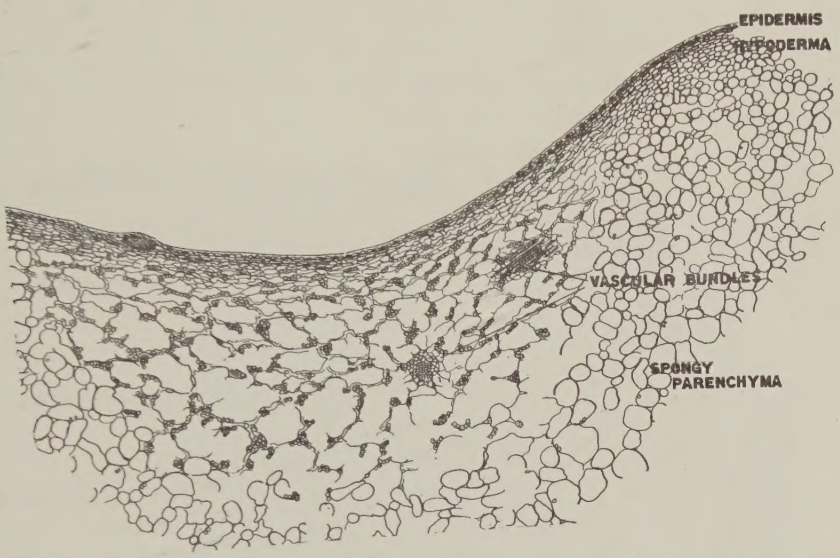

FIG. 4. Section through an apple blotch pit from the Jonathan apple showing the characteristic large, open cavities, the loose broken cell walls in the spongy parenchyma, and starch grains in the cells of the affected region. The living and dead tissues are not separated by a protective layer.

IN THE FOLIAGE

In the Blades

The spots are sunken on the upper surface, and in transverse section the thickness of the leaf is reduced usually to about one-half of the normal (Fig. 1 A). The bundles seem to offer some obstruction to the progress of the fungus. The healthy and dead areas are usually separated by a compact mass of parenchyma cells reaching across the mesophyll, near a vascular bundle. The usually solitary pycnidium occupies a central position on the upper surface of the spot (Plate XXXI, fig. A).

The mycelium is intercellular and ramifies loosely among the palisade tissues and parenchyma cells. It appears to be most extensively developed

below the upper epidermis from which strands extend among the cells of the mesophyll. Infection is followed by the development of a mass of mycelium directly below the epidermis and in the palisade tissue which leads to the formation of the pycnidium. As the pycnidium develops, the dead palisade cells are crushed and pushed aside and the epidermis is raised and ruptured. The upper epidermal and palisade cells collapse as the fungus advances and later the cells of the spongy parenchyma and lower epidermis succumb. The palisade cells become distorted and hang loosely from the dead collapsed epidermis, while those of the spongy parenchyma separate, or are grouped in small masses about the vascular bundles. Occasionally, portions of the lower epidermis and spongy parenchyma fall away, exposing the palisade cells above.

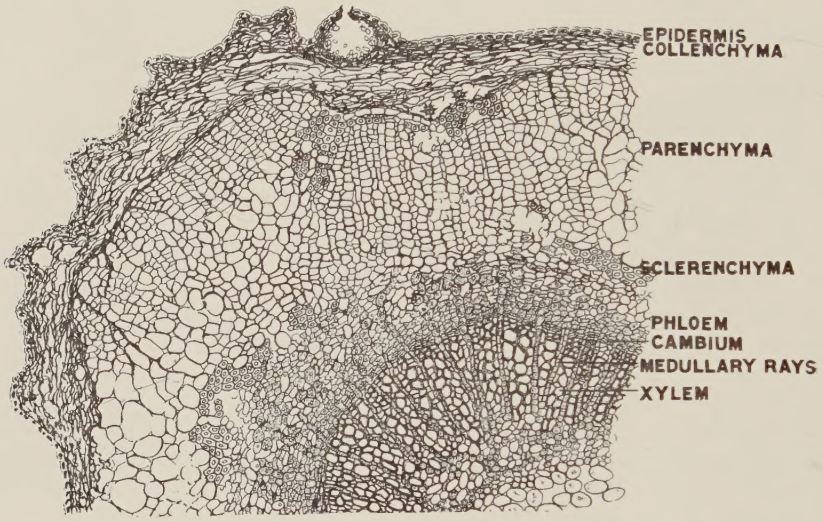

FIG. 5. Section through a petiole showing the effect of the fungus upon the tissues, the dead outer region of the cortex, the abscission layer and the dense columnar arrangement of the parenchyma cells beneath where marked hyperplasia is evident. Strands of sclerenchyma have been pushed out along the abscission layer.

In the Petioles

In the normal apple petiole, the epidermis surmounts a band of collenchyma of two or three compact tiers of cells. Beyond the collenchyma lies a rather broad region of spongy parenchyma the latter bordering on the inside scattered strands of sclerenchyma and phloem. The larger terminating cells of the medullary rays are evident out beyond the phloem.

The diseased region of the petiole is sunken and the surface becomes irregular, dry, and leathery (Fig. 5). In transverse section the epidermal

and collenchyma cells are collapsed. The dead cells are arranged compactly and distorted irregularly. In the spongy parenchyma, the collapse and distortion of the cells is also evident and the cell walls commonly are broken. The necrotic and living tissues of the cortex are definitely separated at a point about midway between the epidermis and the pericycle. The abscission region is sometimes interrupted by strands of sclerenchyma. Here as in the pedicel the protective layer is formed at some distance outside of the sclerenchyma. Underlying the abscission region, in places the cork cambium has given rise to a dense region of parenchyma of small, uniform-sized cells, the phelloderm, arranged in compact, radical layers. In other places the division of the parenchyma cells of the pericycle is evident and appears to account for the presence of strands of sclerenchyma out in the abscission region (Fig. 5). The host is unable to resist the progress of the fungus tangentially and as the result, the epidermis, collenchyma and outer parenchyma may undergo necrosis in this direction until the petiole is girdled. Usually the medullary rays and the vascular elements are influenced by the changes which take place in consequence of which they become somewhat asymmetrically arranged. The same general changes accompany the necrosis of the mid-veins of the leaves (Plate XXXI, fig. C).

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DESCRIPTION OF PLATES

PLATE XXX

FIG. A. Photomicrograph of section through a canker from a water sprout showing the marked response of the cambium to the presence of the fungus. The dense protective layer is apparent directly below the strands of sclerenchyma. From material collected December, 1920.

FIG. B. Section through a water sprout showing the arrangement and construction of the normal tissues. From material collected November 20, 1919.

FIG. C. Photomicrograph of section through a canker at the margin showing the wedge-shaped crack directly outside of the periderm. The cork cambium and phelloderm are very much in evidence. From material collected December, 1920.

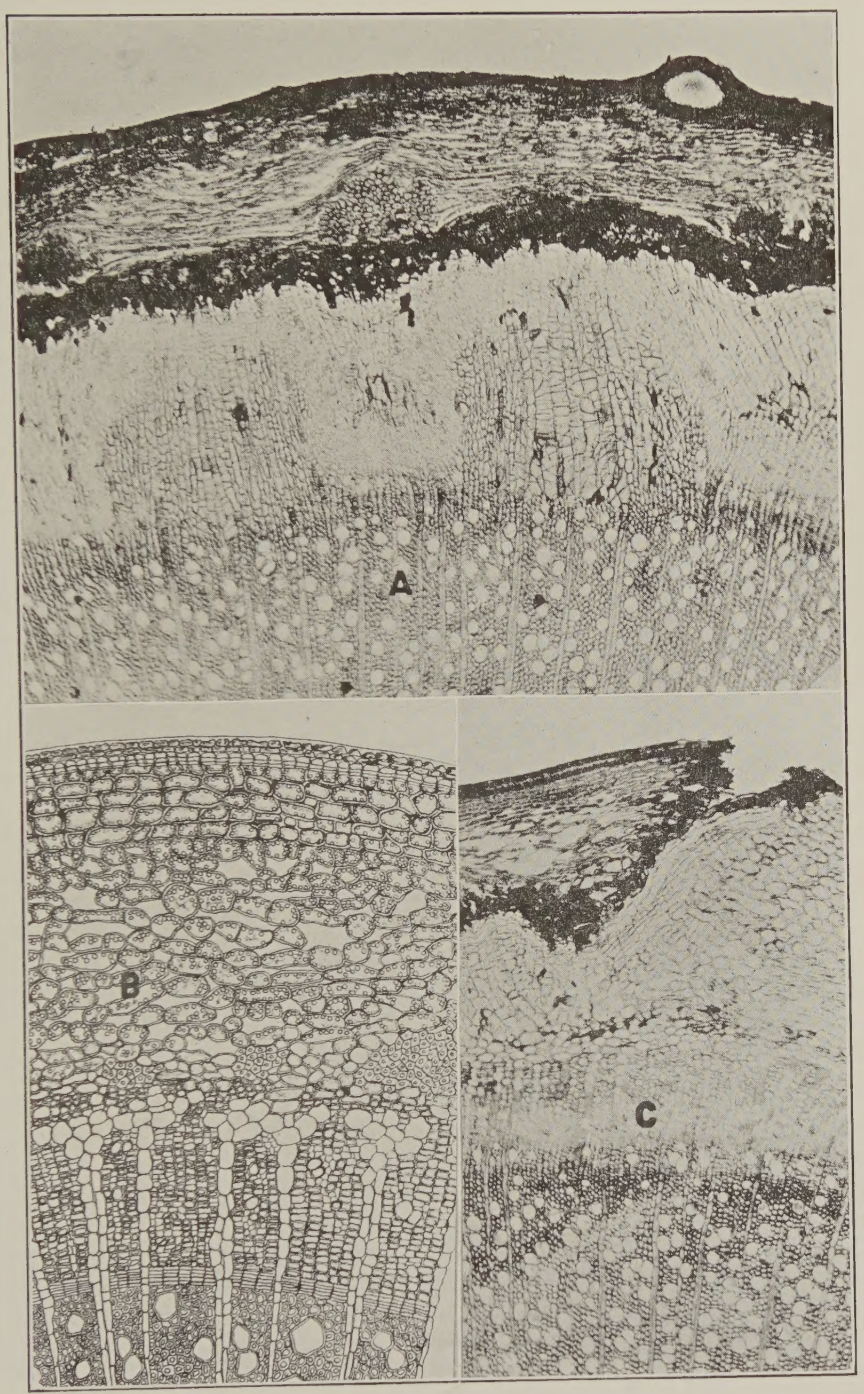
PLATE XXXI

FIG. A. Enlarged view of a portion of an apple leaf affected with *P. solitaria* showing the central position of the usually solitary pycnidium on pale spots of definite sizes and shapes.

FIG. B. Photomicrograph of a section through a spur canker from a three-year-old twig collected June 2, 1921. The dead cankered area is almost exfoliated and the periderm layer below, formed in the season when infection occurred, has eventually become exposed.

FIG. C. Photomicrograph of a section through an affected mid-vein of an apple leaf showing the sunken irregular surface of the lesion, the abscission layer between healthy and diseased areas, and the compact columnar arrangement of small parenchyma cells beneath.

FIG. D. Apple blotch canker on seven-year-old branch of a Duchess apple tree. Exfoliation of the canker is almost complete yet the fungus still persists in the margin.



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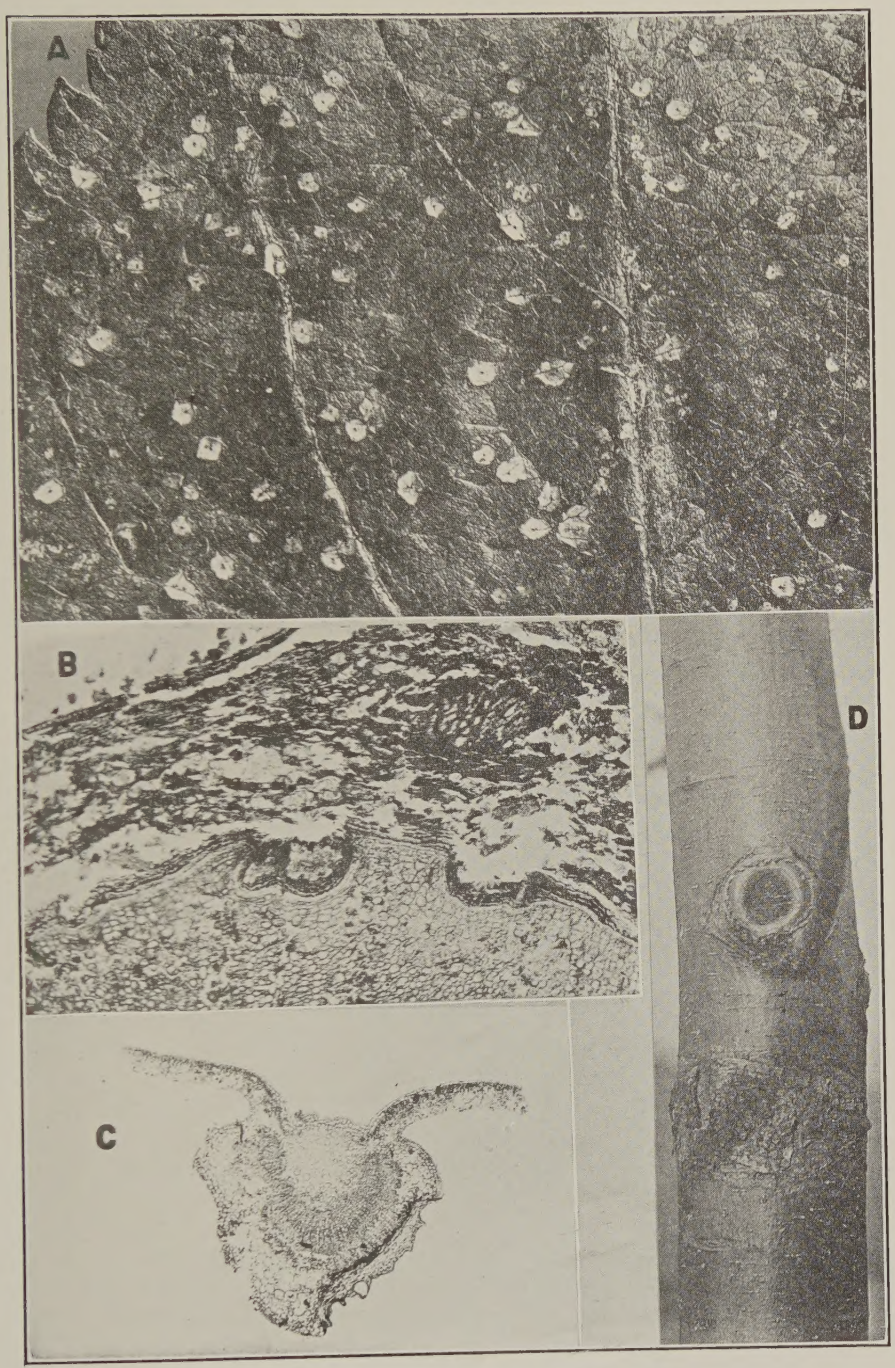

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